

<b>Notice of References Cited</b>	Application/Control No. 09/825,989	Applicant(s)/Patent Under Reexamination FAHEY ET AL.	
	Examiner Cybille Delacroix-Muirheid	Art Unit 1614	Page 1 of 1


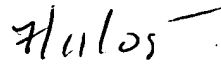
## U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,290,578	03-1994	Passey et al.	426/417
	B	US-			
	C	US-			
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## FOREIGN PATENT DOCUMENTS

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US96/13262 <b>(22) International Filing Date:</b> 16 August 1996 (16.08.96)  <b>(30) Priority Data:</b> 60/002,578           21 August 1995 (21.08.95)   US 08/601,316        16 February 1996 (16.02.96)   US  <b>(71) Applicant:</b> THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US).  <b>(72) Inventors:</b> NODA, Isao; 1568 Hunter Road, Fairfield, OH 45014 (US). SCHECHTMAN, Lee, Arnold; 2069 Edinburg Lane, Fairfield, OH 45014 (US).  <b>(74) Agents:</b> REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SOLVENT EXTRACTION OF POLYHYDROXY-ALKANOATES FROM BIOMASS  <b>(57) Abstract</b> <p>The present invention relates to a process for separating polyhydroxyalkanoate from a biomass, the process comprising extracting the polyhydroxyalkanoate with at least one PHA solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate, <math>\beta</math>-butyrolactone, <math>\gamma</math>-butyrolactone, diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, 1,4-dioxane, tetrahydrofuran, toluene, xylene, and mixtures thereof.</p>		

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## SOLVENT EXTRACTION OF POLYHYDROXY- ALKANOATES FROM BIOMASS

### FIELD

The present invention relates to methods of extracting specific components from other biomass components. More specifically, the present invention relates to the extraction of a polyhydroxyalkanoate from a biological system, such as a plant or bacteria, by performing the extraction with a solvent.

### BACKGROUND

Commodity polymers are typically produced from petrochemical sources by well-known synthetic means. However, recent advances in technology have resulted in the promise of new sources of commodity polymers. Particularly promising is the production of plastic resins using living organisms ("bioplastic"), including genetically manipulated bacteria and crop plants, which are designed to produce polymers such as polyhydroxyalkanoate (PHA); a number of bacteria which naturally produce PHA are also promising sources of PHA. (See for example, Poirier, Y., D.E. Dennis, K. Klomparens and C. Somerville, "Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants", Science, Vol. 256, pp. 520-523 (1992); World Patent Application Publication No. 95/05472, published February 23, 1995; and World Patent Application Publication No. 93/02187, published February 4, 1993; Novel Biodegradable Microbial Polymers, E.A. Dawes, ed., NATO ASI Series, Series E: Applied Sciences - Vol. 186, Kluwer Academic Publishers (1990)). In a large scale production, for example agricultural production, the harvesting and purifying of such bioplastic from the biomass debris is a critical step for determining the practical feasibility of such technology.

The separation of polymeric lipids such as PHA from a large-scale biological source, such as an agricultural crop, is not a trivial task. The conventional separation methods used extensively in the extraction of low molecular weight lipids are not practical to employ in a resin isolation process. For example, a simple mechanical press is impractical because, unlike separating vegetable oils from oilseeds, solid plastics cannot be squeezed out of crops by mechanical pressing.

Separation of PHA by sedimentational methods should be, in principle, possible. However, simple gravitational (1-G force) settling in a

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liquid suspending medium is, in fact, quite impractical. The rate of settling is extremely slow. In addition, such slow settling is easily disrupted by the Brownian motion of the fine PHA particles induced by the thermal fluctuation of the suspending fluid molecules surrounding the particles. Furthermore, the extended period of time required to settle very fine PHA particles introduces the problem of bacterial contamination and subsequent biodegradation of the particle suspension.

Known solvent extraction methods are also impractical for a large-scale separation of PHA from an agricultural crop. A commonly used solvent for the extraction of PHA from bacteria is chloroform. Also described for use are other halogenated hydrocarbon solvents such as dichloromethane, dichloroethane and chloropropane (see, e.g., U.S. Patent 4,562,245, Stageman, issued December 31, 1985; U.S. Patent 4,324,907, Senior, Wright and Alderson, issued April 13, 1982; U.S. Patent 4,310,684, Vanlautem and Gilain, issued January 12, 1982; U.S. Patent 4,705,604, Vanlautem and Gilain, issued November 10, 1987; European Patent Application 036 699, Holmes and Wright, published September 3, 1981; and German Patent Application 239 609, Schmidt, Schmiechen, Rehm and Trennert, published January 10, 1986). However, such solvents are potentially harmful to health and environment. Consequently, the use of a large amount of such solvents, especially near the harvesting site, would be undesirable.

Based on the foregoing, there is a need for a simple and economical process for recovering bioplastics from a large-scale biological source. Such a process would preferably be easily adaptable as an integral part of the agricultural production of related commodities, e.g., oil and meal in the case of oilseeds.

It is therefore an object of the present invention to provide a process for recovering bioplastics from a biomass.

These and other objects of the present invention will become evident to those skilled in the art from a reading of the present disclosure with the appended claims.

#### SUMMARY

A process for separating polyhydroxyalkanoate from a biomass, the process comprising extracting the polyhydroxyalkanoate with at least one PHA solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate,  $\beta$ -butyrolactone,  $\gamma$ -butyrolactone,

diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, tetrahydrofuran, toluene, xylene, and mixtures thereof.

Such a process satisfies the need for a relatively simple, environmentally benign and economical process for recovering bioplastics from a large-scale biological source.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 sets forth a schematic of an embodiment of the invention comprising a one-stage extraction of oil and PHA followed by the stripping of solvent.

Figure 2 sets forth a schematic of an embodiment of the invention comprising a one-stage extraction of oil and PHA followed by a PHA precipitant-induced precipitation of PHA.

Figure 3 sets forth a schematic of an embodiment of the invention comprising a two-stage sequential extraction of oil and PHA.

#### **DETAILED DESCRIPTION**

The following is a list of definitions for terms used herein.

"Alkenyl" means a carbon-containing chain, preferably from about C<sub>2</sub> to about C<sub>24</sub>, more preferably from about C<sub>2</sub> to about C<sub>19</sub>; which may be straight, branched or cyclic, preferably straight or branched, more preferably straight; substituted (mono- or poly-) or unsubstituted; and monounsaturated (i.e., one double or triple bond in the chain), or polyunsaturated (i.e., two or more double bonds in the chain, two or more triple bonds in the chain, or one or more double and one or more triple bonds in the chain), preferably monounsaturated.

"Alkyl" means a carbon-containing chain, preferably from about C<sub>1</sub> to about C<sub>24</sub>, more preferably from about C<sub>1</sub> to about C<sub>19</sub>; which may be straight, branched or cyclic, preferably straight or branched, more preferably straight; substituted (mono- or poly-) or unsubstituted; and saturated.

"Comprising" means that other steps and other ingredients which do not affect the end result can be added. This term encompasses the terms "consisting of" and "consisting essentially of".

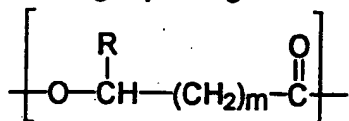
"Extracting polyhydroxyalkanoate from a biomass", in addition to referring to the extraction of the particular PHA produced by a biomass which produces a single PHA, also refers to the extraction of one or more types of PHA when the biomass produces more than one type of PHA.

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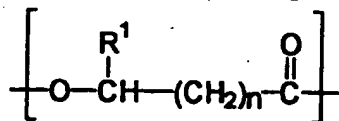
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"Polyhydroxyalkanoate" and "PHA" mean a polymer comprising the following repeating unit:

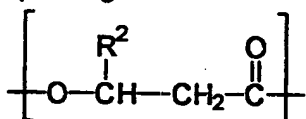


wherein R is preferably H, alkyl or alkenyl; and m is from about 1 to about 4. The terms polyhydroxyalkanoate and PHA include polymers containing one or more different repeating units.

PHAs extractable by the process of the present invention preferably have a melt temperature ("T<sub>m</sub>") of about 80°C or higher. Preferably, such PHAs comprise at least two randomly repeating monomer units, wherein the first randomly repeating monomer unit has the structure



wherein R<sup>1</sup> is H, or C<sub>1</sub> to C<sub>2</sub> alkyl; and n is 1 or 2; the second randomly repeating monomer unit has the structure



wherein R<sup>2</sup> is C<sub>3</sub> to C<sub>19</sub> alkyl or C<sub>3</sub> to C<sub>19</sub> alkenyl; and wherein at least 50% of the randomly repeating monomer units have the structure of the first randomly repeating monomer unit. More preferably, examples of high crystallinity PHAs extractable by the present process include those disclosed in U.S. Patent Application Serial No. 08/ 465,046, Noda, filed June 6, 1995; U.S. Patent Application Serial No. 08/422,008, Noda, filed April 13, 1995; U.S. Patent Application Serial No. 08/422,009, Noda, filed June 5, 1995; U.S. Patent Application Serial No. 08/467,373, Noda, filed June 6, 1995; U.S. Patent Application Serial No. 08/188,271, Noda, filed January 28, 1994; U.S. Patent Application Serial No. 08/469,969, Noda, filed June 6, 1995; U.S. Patent Application Serial No. 08/472,353, Noda, filed June 7, 1995; U.S. Patent Application Serial No. 08/469,269, Noda, filed June 6, 1995; and U.S. Patent 5,292,860, Shiotani and Kobayashi, issued March 8, 1994.

"Solvent" means a substance capable of dissolving another substance (solute) to form a uniformly dispersed mixture (solution) at the molecular or ionic size level.

"Nonsolvent" means a substance which is incapable of appreciably dissolving another substance.

"Precipitant" means a substance which is capable of inducing the precipitation of another substance and/or weakening the solvating power of a solvent. While a precipitant is also considered a nonsolvent, however, a nonsolvent is not always a precipitant. For example, methanol and hexane are PHA precipitants and PHA nonsolvents; whereas oil is a PHA nonsolvent, but not a very effective PHA precipitant (though at extremely high concentrations, oil will cause PHA to precipitate out of solution).

All percentages are by mole % of total composition unless specifically stated otherwise.

All ratios are weight ratios unless specifically stated otherwise.

The present invention, in its product and process aspects, is described in detail as follows.

#### Biomass

Sources from which PHA is extracted via the process of the present invention include single-cell organisms such as bacteria or fungi and higher organisms such as plants (herein collectively referred to as "biomass"). While such biomass could be wild-type organisms, they are preferably genetically manipulated species specifically designed for the production of a specific PHA of interest to the grower. Such genetically manipulated organisms are produced by incorporating the genetic information necessary to produce one or more types of PHA. Typically, such genetic information is derived from bacteria which naturally produce PHA.

Plants useful in the present invention include any genetically engineered plant designed to produce PHA. Preferred plants include agricultural crops such as cereal grains, oilseeds and tuber plants; more preferably, avocado, barley, beets, broad bean, buckwheat, carrot, coconut, copra, corn (maize), cottonseed, gourd, lentils, lima bean, millet, mung bean, oat, oilpalm, peas, peanut, potato, pumpkin, rapeseed (e.g., canola), rice, sorghum, soybean, sugarbeet, sugar cane, sunflower, sweetpotato, tobacco, wheat, and yam. Such genetically altered fruit-bearing plants useful in the process of the present invention include, but are not limited to, apple, apricot, banana, cantaloupe, cherries, grapes, kumquat, lemon, lime, orange, papaya, peaches, pear, pineapple, tangerines, tomato, and watermelon. Preferably the plants are genetically engineered to produce PHA pursuant to the methods disclosed in Poirier, Y., D.E. Dennis, K.



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Klomprens and C. Somerville, "Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants", Science, Vol. 256, pp. 520-523 (1992); World Patent Application Publication No. 95/05472, published February 23, 1995; and World Patent Application Publication No. 93/02187, published February 4, 1993. Particularly preferred plants are soybean, potato, corn and coconut plants genetically engineered to produce PHA; more preferably, soybean.

Bacteria useful in the present invention include any genetically engineered bacteria designed to produce PHA, as well as bacteria which naturally produce PHA. Examples of such bacteria include those disclosed in Novel Biodegradable Microbial Polymers, E.A. Dawes, ed., NATO ASI Series, Series E: Applied Sciences - Vol. 186, Kluwer Academic Publishers (1990); U.S. Patent 5,292,860, Shiotani and Kobayashi, issued March 8, 1994; U.S. Patent 5,250,430, Peoples and Sinskey, issued October 5, 1993; U.S. Patent 5,245,023, Peoples and Sinskey, issued September 14, 1993; U.S. Patent 5,229,279, Peoples and Sinskey, issued July 20, 1993.

#### Solvent Extraction

The present invention relates to a process for separating polyhydroxyalkanoate from a biomass, the process comprising extracting the polyhydroxyalkanoate with at least one PHA solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate,  $\beta$ -butyrolactone,  $\gamma$ -butyrolactone, diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, 1,4-dioxane, tetrahydrofuran, toluene, xylene, and mixtures thereof. Preferably, the PHA solvent is acetone, acetonitrile,  $\gamma$ -butyrolactone, 1,4-dioxane, methyl acetate, toluene, methyl ethyl ketone, ethyl acetate or mixtures thereof. More preferably the PHA solvent is acetone or ethyl acetate; more preferably still, acetone.

PHA precipitants may also be employed at certain stages in the process to affect the precipitation of PHA. Use of such precipitants in the process is discussed in more detail below. Preferred PHA precipitants include, but are not limited to, butane, diethyl ether, diglyceride, ethanol, fat, heptane, hexane, methanol, monoglyceride, octane, oil, pentane, triglyceride, water, or mixtures thereof.

Preferably the PHA solvent is employed in the process at an elevated temperature, as the rate of dissolution of PHA in the PHA solvent at

elevated temperatures has been found to be substantially faster. While extraction of the PHA may be conducted between about 20°C and the melt temperature of the PHA; more preferably between about 20°C and about 80°C; more preferably between about 45° and the boiling point of the PHA solvent; more preferably still, from about 50° to about 60°C.

Preferably the solid mass containing the PHA is stirred during extraction with the PHA solvent, as this also accelerates the rate of dissolution of the PHA.

The removal of the PHA solvent from a solution containing PHA results in the eventual precipitation of PHA as crystalline solids. In the process of stripping the solvent, however, the concentrated PHA solution often forms a very high viscosity fluid or sometimes even a gel; which can be extremely difficult to process. If the solution contains a relatively nonvolatile PHA nonsolvent in addition to the PHA solvent and PHA, the PHA will precipitate upon the removal of the PHA solvent and form a suspension in the PHA nonsolvent.

a. One-stage extraction of oil and PHA followed by the stripping of solvent

In one embodiment of the invention, the process comprises a) treating a biomass comprising PHA and oil (e.g., plant material) with the PHA solvent; b) removing any insoluble plant material, thereby leaving behind a solution of polyhydroxyalkanoate and oil dissolved in the PHA solvent; c) removing the PHA solvent from the solution, thereby resulting in precipitation of the polyhydroxyalkanoate in the oil; and d) removing the oil, thereby leaving behind the polyhydroxyalkanoate.

This embodiment is exemplified in schematic form in Figure 1. This embodiment provides the simplest method of extracting both oil and PHA from a biomass containing these components. The biomass (e.g., soybean containing oil and PHA) is exposed to the PHA solvent. After separation of the solid biomass, the PHA solvent is then directly stripped away from the resultant solution containing the extracted oil and PHA. Since PHAs having a T<sub>m</sub> of about 80°C or higher are usually insoluble in oil, the removal of the solvent results in the precipitation of PHA in oil. The oil and PHA are subsequently separated by conventional means, such as filtration.

b. One-stage extraction of oil and PHA followed by nonsolvent-induced precipitation

In another embodiment of the invention, the process comprises a) treating a biomass comprising PHA and oil (e.g., plant material) with the

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PHA solvent; b) removing any insoluble plant material, thereby leaving behind a solution of polyhydroxyalkanoate and oil dissolved in the PHA solvent; c) treating the solution with a PHA precipitant, thereby resulting in precipitation of the polyhydroxyalkanoate; d) removing the PHA precipitant and the oil dissolved therein, thereby leaving behind the polyhydroxyalkanoate. Optionally, the PHA solvent and the PHA precipitant may be subsequently stripped from the oil.

This embodiment is exemplified in schematic form in Figure 2. This embodiment exposes the biomass (e.g., soybean) containing oil and PHA only once to a PHA solvent, such as acetone, which is a good solvent for both oil and PHA. The addition of a PHA precipitant (e.g., hexane) to the extraction solution containing oil and PHA, results in the precipitation of PHA. The supernatant mixture comprising PHA solvent, PHA precipitant and oil is removed to yield PHA. The PHA solvent and PHA precipitant are subsequently stripped away from the supernatant solution to yield oil. The PHA solvent and PHA precipitant may then be separated from each other and used again.

Preferred PHA precipitants are miscible with the PHA solvent. Preferred PHA precipitants include diethyl ether, diglyceride, ethanol, fat, heptane, hexane, methanol, monoglyceride, oil, triglyceride, water, or mixtures thereof. More preferred precipitants are those which are miscible in oil, such as diethyl ether, diglyceride, ethanol, fat, heptane, hexane, methanol, monoglyceride, oil, triglyceride, or mixtures thereof; more preferably hexane and methanol.

c. Two-stage sequential extraction of oil and PHA

In another embodiment of the invention, the process comprises a) treating the biomass comprising PHA and oil (e.g., plant material) with an oil solvent that does not dissolve the polyhydroxyalkanoate; b) removing the oil solvent and the oil dissolved therein, thereby leaving behind any insoluble plant material; c) treating the oil-solvent-treated biomass (i.e., defatted biomass) with the PHA solvent; d) removing any insoluble plant material, thereby leaving behind a solution of PHA solvent and PHA dissolved therein; and e) stripping the PHA solvent from the solution, thereby leaving behind PHA.

This embodiment is exemplified in schematic form in Figure 3. The biomass (e.g., soybean) is first exposed to an oil solvent (e.g., hexane) which does not dissolve PHA. Consequently, the first stage only extracts

the oil portion. The oil solvent is then stripped from the oil to be used again for further oil extraction. The dried defatted soybean is then exposed to a PHA solvent. The second extraction stage removes PHA from the defatted soybean. The PHA solvent is then stripped from the PHA extraction solution, leaving solid PHA.

Preferred oil solvents include butane, diethyl ether, diglyceride, ethanol, fat, heptane, hexane, methanol, monoglyceride, octane, oil (with a different grade than the oil being extracted), pentane, triglyceride, or mixtures thereof. Preferably, the oil solvent is more volatile than the oil being extracted, thereby facilitating its subsequent stripping from the extracted oil. More preferably, the oil solvent is hexane.

The previously described embodiments of the present invention have many surprising advantages, including the ability to extract crystallizable high melt temperature (about 80°C or higher) PHA without using solvent containing halogens and to effectively co-extract oil simultaneously. The PHA solvents used in this invention such as acetone and ethyl acetate, are inexpensive, safe and benign and readily available, even from renewable sources. Such PHA solvents are also considered far less damaging to the environment, especially the ozone layer of the earth, compared to halogen containing compounds typically used for the extraction of PHAs from bacteria.

The discovery of appropriate solvents for crystalline polymers having relatively high melt temperatures is not a trivial task. Unlike most low molecular weight compounds and noncrystallizable amorphous polymers, the solubility of crystalline polymers cannot be predicted from the commonly used simple criteria, such as the similarity of chemical architecture or matching of refractive indices, dielectric constants or solubility parameters. A good example of the inability to predict the solubility of crystalline polymers is the well-known insolubility of linear polyethylene in hexane, where both compounds are made of the identical hydrocarbon repeat units. Similarly, crystalline aliphatic polyesters like isotactic poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) are not appreciably soluble in ethyl acetate or acetone, even though the chemical structures of these compounds may suggest certain molecular affinity. Thus, the fortuitous discovery that crystalline PHA containing a small amount of medium size branches can be readily dissolved in such solvents is indeed surprising.

Additional surprising advantages are realized when the extraction of PHA is carried out in conjunction with the extraction of oil. The oil initially serves as a miscible co-solvent to promote the extraction of PHA. However, upon removal of a volatile PHA solvent, the oil will later become an effective suspending medium for the precipitating PHA due to its limited PHA-solvating power. Compared to a concentrated solution, a suspension of polymer solid in a fluid nonsolvent typically has much lower apparent viscosity and thus possesses superior processability. The elimination of the serious problem of gelation often encountered during the stripping of solvent from a concentrated polymer solution is an additional unexpected and significant advantage of the invention.

The following examples further describe and demonstrate the preferred embodiments within the scope of the present invention. The examples are given solely for the purpose of illustration, and are not to be construed as limitations of the present invention since many variations thereof are possible without departing from its spirit and scope.

#### EXAMPLE 1

##### Extraction Followed by Solvent Stripping

60 g of soybean sample containing vegetable oil and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) containing 7.5% 3-hydroxyhexanoate repeat units is placed in a closed container charged with 600 mL of acetone containing a very small amount (ca. 0.1 g) of vegetable oil and stirred for 3 hrs at 55°C. The acetone solution containing the extracted oil and PHA is then drained from the soybean using a wire-mesh filter. The extraction solution containing vegetable oil, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and acetone is placed in a steam-heated kettle to boil off the volatile acetone, which is collected by a water-chilled condenser. After the removal of the acetone, solid flakes of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) appear in the remaining oil which are drained by using a fine mesh filter to yield 7 g of pure vegetable oil. The polymer flakes are washed with cold acetone, previously collected by the condenser, to remove the residual entrained oil and then dried to yield 6 g of crystalline solid of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). The acetone used for the washing, which now contains a small amount of oil, is then used for further extraction of oil and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate).

## EXAMPLE 2

### Extraction Followed by Nonsolvent Precipitation

60 g of soybean sample containing vegetable oil and poly(3-hydroxybutyrate-co-3-hydroxyoctadecanoate) containing 5% 3-hydroxyoctadecanoate repeat units is placed in a closed container charged with 600 mL of acetone and stirred for 3 hrs at 55°C. The acetone solution containing the extracted oil and PHA is then drained from the soybean using a wire-mesh filter. To this solution 800 mL of hexane is added to induce the precipitation of poly(3-hydroxybutyrate-co-3-hydroxyoctadecanoate). The polymer flakes are then separated from the supernatant solution with a fine mesh filter and dried to yield 5 g of poly(3-hydroxybutyrate-co-3-hydroxyoctadecanoate). The supernatant solution containing vegetable oil, acetone and hexane is placed in a steam-heated kettle to boil off the volatile mixture of hexane and acetone, which is collected by a water-chilled condenser for further separation to pure hexane and acetone. After the removal of hexane and acetone, 7 g of vegetable oil is collected.

## EXAMPLE 3

### Two-Stage Extraction

60 g of soybean sample containing vegetable oil and poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) containing 6% 3-hydroxyoctanoate repeat units is placed in 600 mL of hexane and stirred for 2 hrs. The hexane solution containing the extracted oil is then drained from the soybean using a wire-mesh filter. The solution is then placed in a steam-heated kettle to boil off the hexane, which is collected by a water-chilled condenser for further use. After the removal of hexane, 6 g of vegetable oil is collected. The defatted soybean is then placed in a closed container charged with 600 mL of acetone and stirred for 3 hours at 55°C. The acetone solution containing PHA is then drained from the soybean using a wire-mesh filter. The solution is then placed in a steam-heated kettle to boil off the acetone, which is collected by a water-chilled condenser for further use. After the removal of acetone, 6 g of solid crystalline poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) is collected.

All publications, issued patents and patent applications mentioned hereinabove are hereby incorporated in their entirety by reference.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or

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**PCT/US96/13262**

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**changes in light thereof will be suggested to one skilled in the art and are to be included in the spirit and purview of this application and scope of the appended claims.**



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1. A process for separating polyhydroxyalkanoate from a biomass comprising the polyhydroxyalkanoate, the process characterized by extracting the polyhydroxyalkanoate with at least one polyhydroxyalkanoate solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate,  $\beta$ -butyrolactone,  $\gamma$ -butyrolactone, diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, 1,4-dioxane, tetrahydrofuran, toluene, xylene and mixtures thereof; wherein the polyhydroxyalkanoate has a melt temperature of about 80°C or higher.

2. A process according to claim 1 wherein the biomass is bacteria or plant material.

3. A process according to any one of the preceding claims wherein the plant material further comprises oil characterized by:

- a) treating the plant material with the polyhydroxyalkanoate solvent;
- b) removing any insoluble plant material, thereby leaving behind a solution of polyhydroxyalkanoate and oil dissolved in the polyhydroxyalkanoate solvent;
- c) removing the polyhydroxyalkanoate solvent from the solution, thereby resulting in precipitation of the polyhydroxyalkanoate in the oil; and
- d) removing the oil, thereby leaving behind the polyhydroxyalkanoate.

4. A process according to any one of the preceding claims wherein the plant material further comprises oil characterized by:

- a) treating the plant material with the polyhydroxyalkanoate solvent;
- b) removing any insoluble plant material, thereby leaving behind a solution of polyhydroxyalkanoate and oil dissolved in the polyhydroxyalkanoate solvent;
- c) treating the solution with a polyhydroxyalkanoate precipitant, thereby resulting in precipitation of the polyhydroxyalkanoate;



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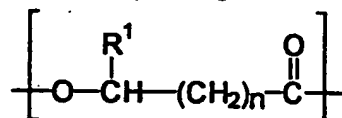
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- d) removing the polyhydroxyalkanoate precipitant, the polyhydroxyalkanoate solvent and the oil dissolved therein, thereby leaving behind the polyhydroxyalkanoate;
- e) stripping the polyhydroxyalkanoate solvent and the polyhydroxyalkanoate precipitant from the oil, thereby leaving behind polyhydroxyalkanoate.

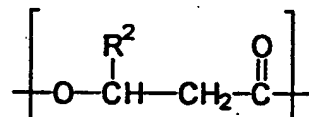
5. A process according to any one of the preceding claims wherein the plant material further comprises oil characterized by:

- a) treating the plant material with an oil solvent that does not dissolve the polyhydroxyalkanoate;
- b) removing the oil solvent and the oil dissolved therein;
- c) treating the biomass with the polyhydroxyalkanoate solvent;
- d) removing any insoluble plant material, thereby leaving behind a solution of polyhydroxyalkanoate solvent and polyhydroxyalkanoate dissolved therein; and
- e) stripping the polyhydroxyalkanoate solvent from the solution, thereby leaving behind polyhydroxyalkanoate.

6. A process according to any one of the preceding claims wherein the polyhydroxyalkanoate comprises at least two randomly repeating monomer units, wherein the first randomly repeating monomer unit has the structure



wherein R<sup>1</sup> is H or C<sub>1</sub> to C<sub>2</sub> alkyl, and n is 1 or 2; the second randomly repeating monomer unit has the structure



wherein R<sup>2</sup> is C<sub>3</sub> to C<sub>19</sub> alkyl or C<sub>3</sub> to C<sub>19</sub> alkenyl; and wherein at least 50% of the randomly repeating monomer units have the structure of the first randomly repeating monomer unit.

7. A process according to any one of the preceding claims wherein the PHA solvent is acetone or ethyl acetate.

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8. A process according to any one of the preceding claims wherein the polyhydroxyalkanoate precipitant is selected from the group consisting of diethyl ether, ethanol, fat, heptane, hexane, methanol, oil, triglyceride, water and mixtures thereof.

9. A process according to any one of the preceding claims wherein the oil solvent is selected from the group consisting of butane, diethyl ether, diglyceride, ethanol, fat, heptane, hexane, methanol, monoglyceride, octane, oil having a different grade than the oil from the plant material, pentane, triglyceride and mixtures thereof.

10. The polyhydroxyalkanoate extracted by the process according to any one of the preceding claims.

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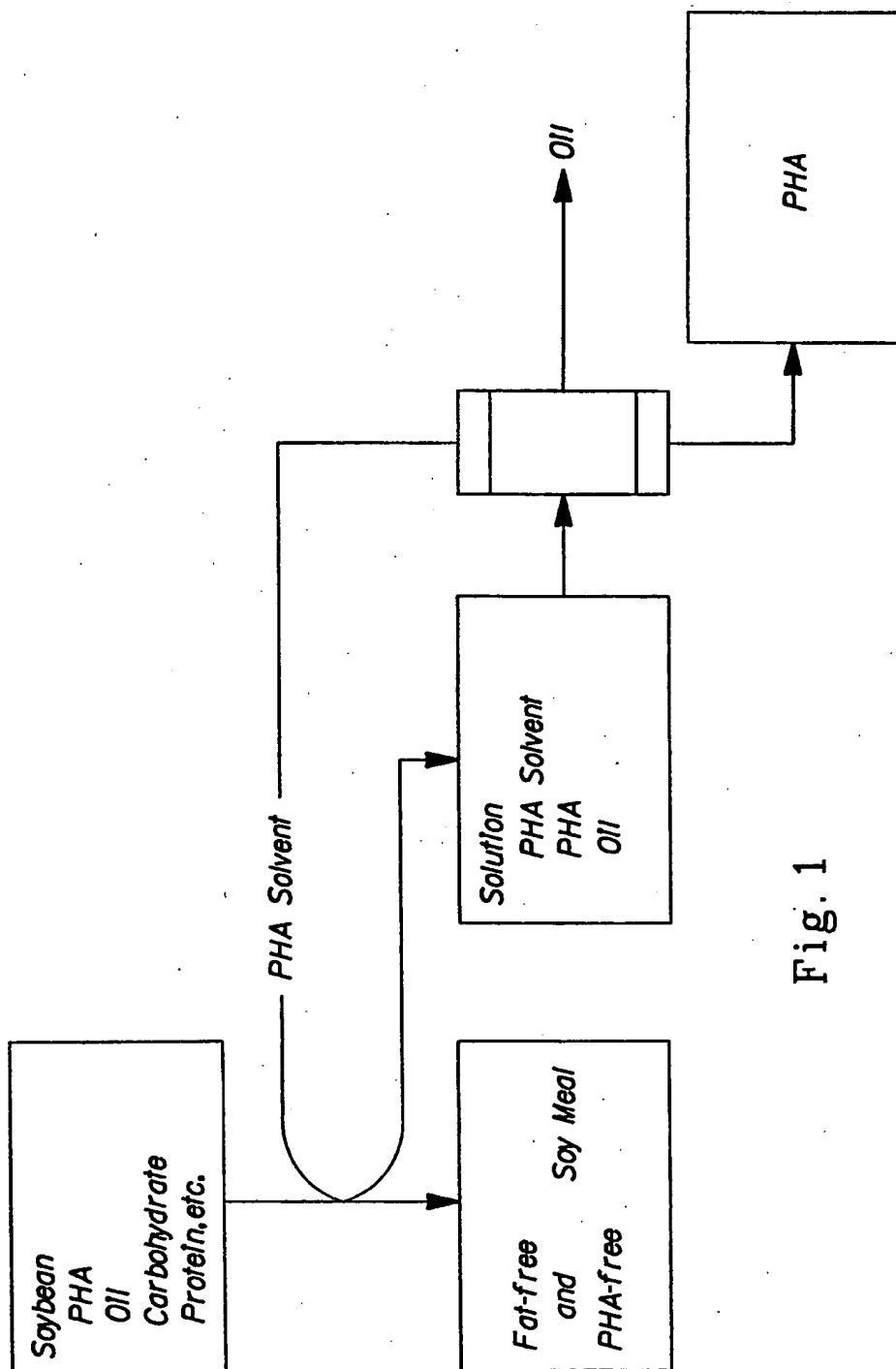
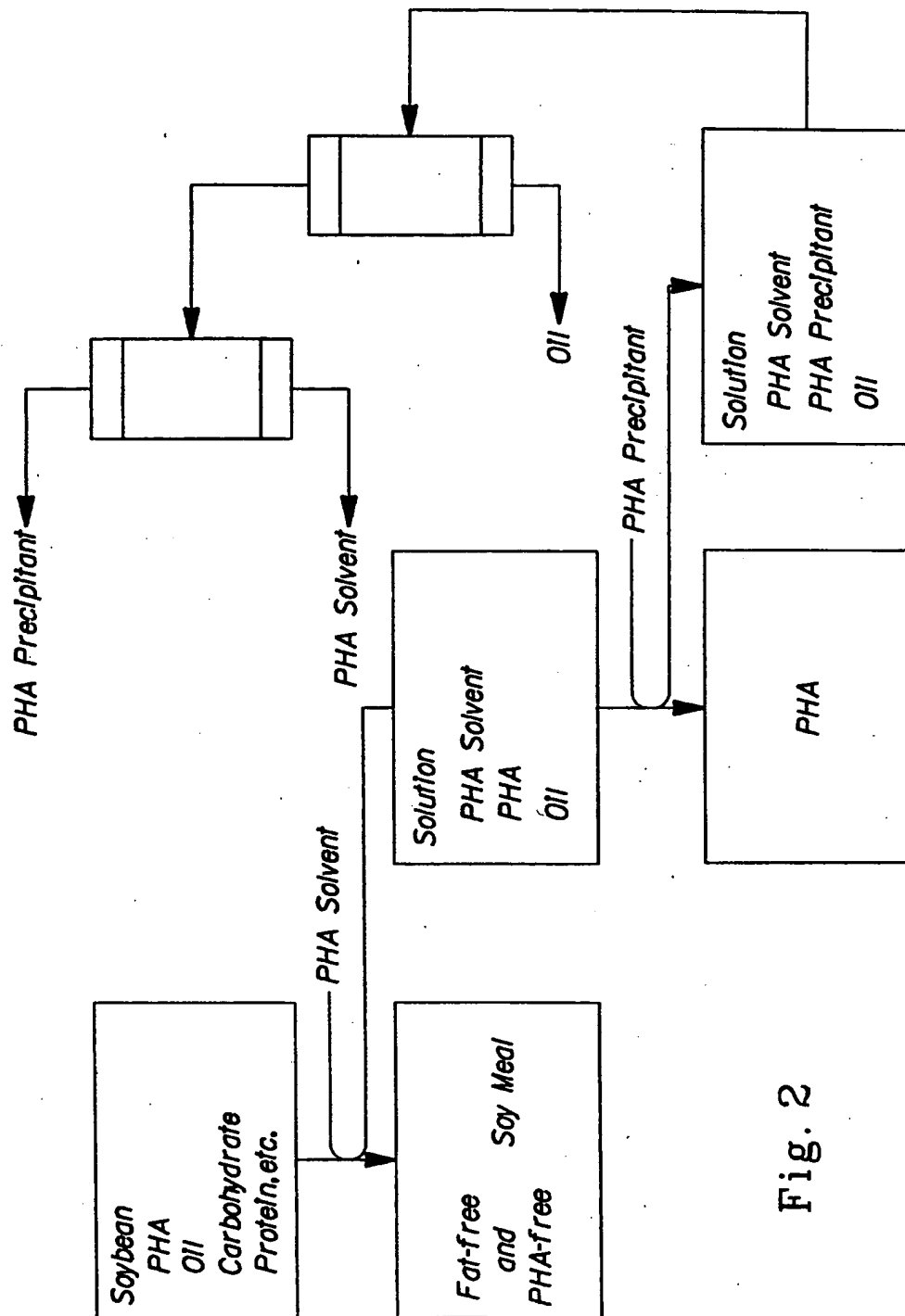


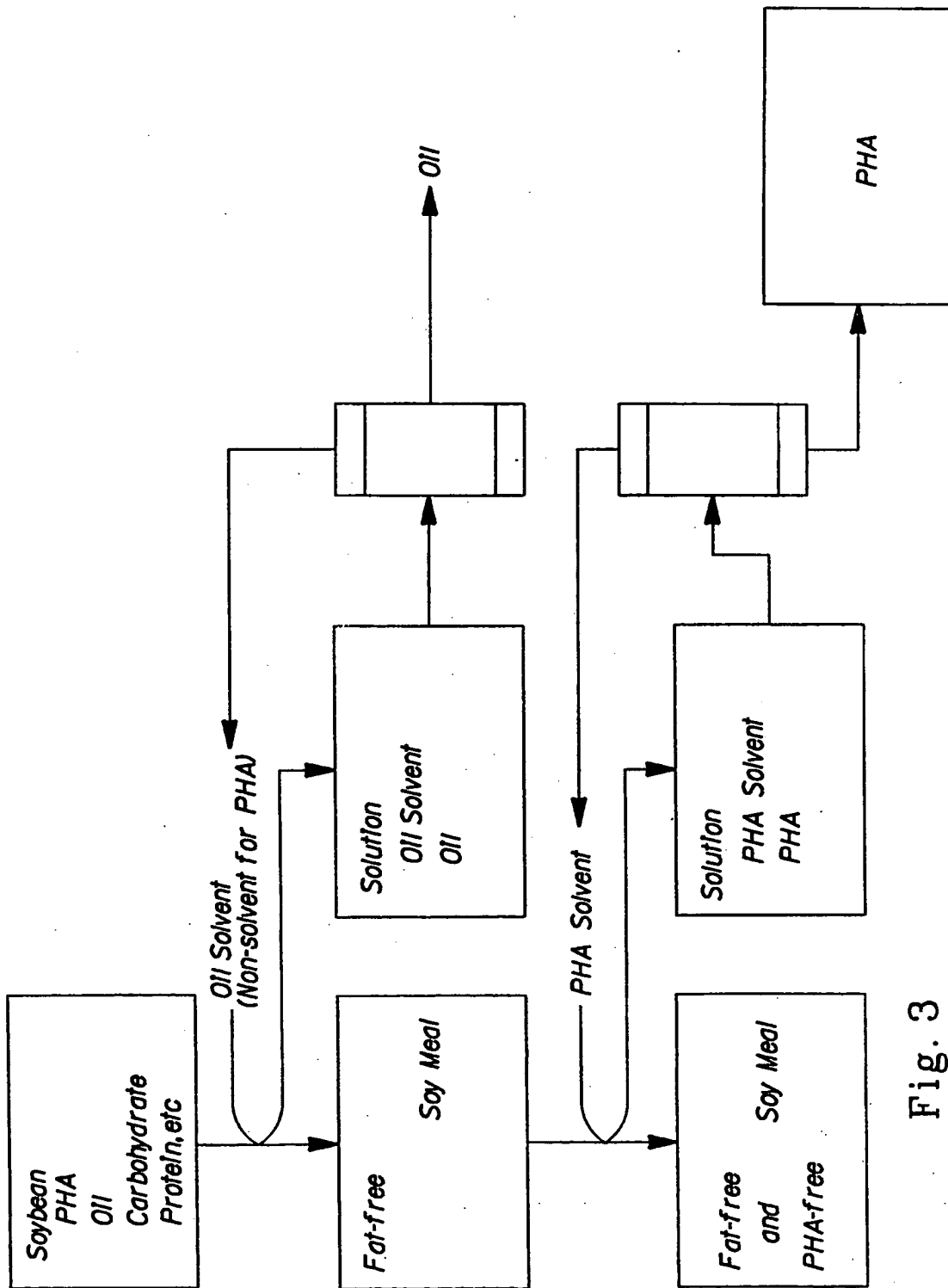
Fig. 1

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## INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/US 96/13262

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12P7/62 C08G63/06 C08G63/89

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 355 397 (DANUBIA PETROCHEMIE) 28 February 1990	1,2,10
Y	see claims	6
X	WO,A,93 11656 (FIRMENICH & CIE ;OHLEYER ERIC (FR)) 10 June 1993	1,2,7,10
Y	see claims	6
X	WO,A,93 23554 (SUNA AG) 25 November 1993	1,2,10
Y	see claims	6
Y	EP,A,0 416 624 (SHOWA DENKO KK) 13 March 1991	6
	see claims	
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

21 November 1996

Date of mailing of the international search report

29. 11. 96

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Delanghe, L

## INTERNATIONAL SEARCH REPORT

Introductory Application No

PCT/US 96/13262

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 533 144 (KANEKAFUCHI CHEMICAL IND) 24 March 1993 see claims & US,A,5 292 860 cited in the application ---	6
A	WO,A,94 11519 (ZENECA LTD ;FENTEM PHILIP ANTHONY (GB)) 26 May 1994 see claims ---	3
A	WO,A,93 02187 (UNIV MICHIGAN) 4 February 1993 cited in the application see claims ---	3
P,X	WO,A,96 06179 (ZENECA LTD ;LIDDELL JOHN MACDONALD (GB)) 29 February 1996 see page 2 - page 3; claims -----	1-3,7,10

Form PCT/ISA/218 (continuation of second sheet) (July 1992)

## INTERNATIONAL SEARCH REPORT

(information on patent family members)

International Application No

PCT/US 96/13262

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0355307	28-02-90	AT-B- 390068 AT-T- 116370 DE-A- 3823754 DE-D- 58908825 ES-T- 2065945 JP-A- 2069187 US-A- 4968611	12-03-90 15-01-95 18-01-90 09-02-95 01-03-95 08-03-90 06-11-90
WO-A-9311656	10-06-93	EP-A- 0569569 JP-T- 6505403 US-A- 5422257	18-11-93 23-06-94 06-06-95
WO-A-9323554	25-11-93	DE-A- 4215860 DE-A- 4215861 DE-A- 4215862 CZ-A- 9402664 EP-A- 0641387 LT-B- 3050 LV-B- 10656 SK-A- 131394	18-11-93 18-11-93 18-11-93 15-03-95 08-03-95 25-10-94 20-02-96 08-11-95
EP-A-0416624	13-03-91	JP-A- 3180186	06-08-91
EP-A-0533144	24-03-93	JP-A- 5093049 US-A- 5292860	16-04-93 08-03-94
WO-A-9411519	26-05-94	AU-A- 5427194	08-06-94
WO-A-9302187	04-02-93	AU-B- 671953 AU-A- 2323892 BE-A- 1006851 BR-A- 9206297 CA-A- 2113412 CZ-A- 9400108 EP-A- 0595896 ES-A- 2046142 FI-A- 940242 FR-A- 2679735 HU-A- 66825 IT-B- 1255348	19-09-96 23-02-93 10-01-95 17-10-95 04-02-93 15-12-94 11-05-94 16-01-94 11-03-94 05-02-93 30-01-95 31-10-95



## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/13262

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9302187		JP-T- 6509233	20-10-94
		NL-A- 9201288	16-02-93
		NO-A- 940157	18-03-94
		NZ-A- 243626	28-03-95
		PT-A- 100705	29-10-93
		SK-A- 6494	05-10-94
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WO-A-9606179	29-02-96	AU-A- 3188395	14-03-96
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## ISSUE SLIP STAPLE AREA (for additional cross references)

POSITION	INITIALS	ID NO.	DATE
FEE DETERMINATION	<i>[Signature]</i>		<i>11-1-01</i>
O.I.P.E. CLASSIFIER			
FORMALITY REVIEW	<i>AK</i>	<i>931</i>	<i>06/30/01</i>
RESPONSE FORMALITY REVIEW			

## INDEX OF CLAIMS

✓ ..... Rejected      N ..... Non-elected  
 " ..... Allowed      I ..... Interference  
 - (Through numeral) ... Canceled      A ..... Appeal  
 + ..... Restricted      O ..... Objected

*164/520*

Claim	Final	Original	Date
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SEARCHED			
Class	Sub.	Date	Exmr.
426	425	5/1/02	cm
↓	429	↓	↓
↓	431	↓	↓
↓	615	↓	↓
updated		1/9/03	cm
up d cited		11/16/03	cm
updated		1-23-05	cm
up d cited		7/8/05	cm
↓		7/11/05	cm

INTERFERENCE SEARCHED			
Class	Sub.	Date	Exmr.

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	Date	Exmr.
EAST	5/1/02	cm
(search history in file)	↓	↓
EAST update	1/9/03	cm
EAST update	11/16/03	cm
search update reviewed library search	1-23-05	cm
inventor name search	7/8/05	cm
EAST update	7/8/05	cm
patentability conference with Chris Low and Paddy	7/8/05	cm
	↓	↓

(RIGHT MARGINS)



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## \*BIBDATASHEET\*

Bib Data Sheet

CONFIRMATION NO. 4463

SERIAL NUMBER 09/825,989	FILING DATE 04/05/2001  RULE	CLASS 514	GROUP ART UNIT 1614	ATTORNEY DOCKET NO. 046585/0138
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APPLICANTS

Jed W. Fahey, Eldersburg, MD;  
 Paul Talalay, Baltimore, MD;

**\*\* CONTINUING DATA \*\*\*\*\***

This application is a DIV of 09/425,890 10/25/1999 PAT 6,242,018  
 which is a DIV of 09/118,867 07/20/1998 PAT 6,177,122  
 which is a DIV of 08/840,234 04/11/1997 PAT 5,968,567

**\*\* FOREIGN APPLICATIONS \*\*\*\*\***

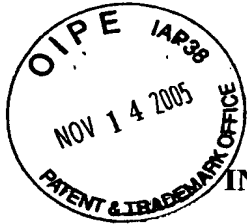
IF REQUIRED, FOREIGN FILING LICENSE GRANTED \*\* SMALL ENTITY \*\*  
 \*\* 05/30/2001

Foreign Priority claimed 35 USC 119 (a-d) conditions met Verified and Acknowledged	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> Met after Allowance Examiner's Signature _____ Initials _____	STATE OR COUNTRY MD	SHEETS DRAWING 2	TOTAL CLAIMS 20	INDEPENDENT CLAIMS 2
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ADDRESS  
 22428  
 FOLEY AND LARDNER  
 SUITE 500  
 3000 K STREET NW  
 WASHINGTON, DC  
 20007

TITLE  
 Cancer chemoprotective food products

FILING FEE  RECEIVED 391	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	<input type="checkbox"/> All Fees <input type="checkbox"/> 1.16 Fees ( Filing ) <input type="checkbox"/> 1.17 Fees ( Processing Ext. of time ) <input type="checkbox"/> 1.18 Fees ( Issue ) <input type="checkbox"/> Other _____
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Atty. Dkt. No. 046585/0138

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: FAHEY et al.

Title: *Cancer Chemoprotective Food Products*

Appl. No.: 09/825,989

Filing Date: April 5, 2001

Examiner: Delacroix Muirheid, Cybille

Art Unit: 1614

**AMENDMENT AND REPLY UNDER 37 CFR 1.111**

**Mail Stop Amendment**  
Commissioner for Patents  
PO Box 1450  
Alexandria, Virginia 22313-1450

Sir:

This communication is responsive to the Non-Final Office Action dated July 14, 2005, concerning the above-referenced patent application.

**Remarks** begin on page 2 of this document.

Please amend the application as follows.

11/15/2005 JADD01 00000115 09825989  
01 FC:2251 60.00 DP

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Atty. Dkt. No. 046585/0138  
Appln. No. 09/825,989

## REMARKS

### **I. Introduction**

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 1-47 and 64 are cancelled. The cancellation of claims does not constitute acquiescence in the propriety of any rejection set forth by the Examiner. Applicant reserves the right to pursue the subject matter of the canceled claims in subsequent divisional applications.

A detailed listing of all claims that are, or were, in the application, irrespective of whether the claims remain under examination in the application, is presented, with an appropriate defined status identifier.

Upon entry of this Amendment, claims 48-63 and 65-71 will remain pending in the application.

Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

### **II. Response to Issues Raised by Examiner in Outstanding Office Action: Claim Rejections - 35 U.S.C. § 103**

Applicant notes that in the previous Office Action dated January 27, 2005, claims 48-57 were indicated to be allowable and claims 58-71 were rejected under 35 U.S.C. § 112, second paragraph. There were no rejections in view of the prior art because Applicant's previously submitted argumentation had successfully overcome all of the Examiner's prior art rejections with respect to Jones et al. (U.S. Patent No. 4,158,656), Pusateri et al. (U.S. Patent No. 5,882,646), Cho et al. (WO 94/19948) and Anjou et al. (U.S. Patent No. 4,083,836). However, on page 2 of the outstanding Office Action, the Examiner states "[h]owever, upon reconsidering of the pending claims with the examiner's supervisor, the following new ground(s) of rejection is respectfully submitted."

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Applicant notes that many of the rejections in the outstanding Office Action are substantially identical to the rejections stated in the January 16, 2003 Office Action. Applicant provided argumentation with respect to these rejections in the July 16, 2003 Amendment. However, the Examiner's rejections in the outstanding Office Action do not address Applicant's argumentation. Applicant respectfully requests that the Examiner provide reasoning as to why Applicant's arguments are not considered sufficient to overcome the rejections of record.

a. Rejection of Claims 48-57 as Being Unpatentable Over  
Jones et al. (USP 4,158,656) in view of WO 97/07230

Claims 48-57 are rejected by the Examiner under 35 U.S.C. § 103 as being obvious over Jones et al. (USP 4,158,656) in view of WO 97/07230. Applicant respectfully requests reconsideration and withdrawal of the rejection.

The Examiner has failed to establish a *prima facie* case of obviousness because all three of the criteria required to establish a *prima facie* case of obviousness have not been met. In order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references (or references when combined) must teach or suggest all the claim limitations. See MPEP 2142.

i. *The cited art fails to teach each and every limitation of the  
claimed invention*

Jones et al. and WO '230 fail to teach or suggest every limitation of the claimed invention. The Examiner asserts that the claimed invention is obvious over the combined teachings of Jones et al. and WO '230 because although Jones et al. do not teach extracting glucosinolates using a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide, WO '230 discloses an extraction method using a mixture of these solvents. Applicant respectfully disagrees.

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The Examiner directs Applicant's attention to the abstract and page 6, first paragraph of WO '230 for the disclosure of an extract method using a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide. However, Applicant notes that these disclosures in WO '230 are directed to a process for separating polyhydroxyalkanoate (PHA) from a biomass with at least one solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate, beta-butyrolactone, gamma-butyrolactone, diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, 1,4-dioxane, tetrahydrofuran, toluene, xylene, and mixtures thereof. The term "and mixtures thereof" covers 2,097,151 possible solvent combinations. However, nowhere in WO '230 is the specific combination of dimethyl sulfoxide, acetonitrile and dimethylformamide taught or suggested. Instead, the Examiner is picking and choosing one specific combination out of a possible 2,097,151. Because WO '230 fails to teach the specific combination of dimethyl sulfoxide, acetonitrile and dimethylformamide, WO '230 does not cure the deficiencies of Jones et al.

ii. *There is no suggestion or motivation to modify the teachings of Jones et al. with the teachings of WO '230*

As discussed above, the combined teachings of Jones et al. and WO '230 fail to teach each and every limitation of the claimed invention. Therefore, the claimed invention is not obvious over the combined teachings of these documents.

However, assuming *arguendo* that the cited references do disclose each and every limitation of the claimed invention, the claimed invention is still not obvious over the cited art because a person of ordinary skill in the art would not have been motivated to combine the teachings of WO '230 with the teachings of Jones et al. to arrive at the claimed invention. The claimed invention is directed to a method of extracting glucosinolates and isothiocyanates from plant tissue. In contrast, WO '230 is directed to a method for separating PHA's from a biomass. While WO '230 may state that the source of the biomass may be plants, such as rapeseed (see page 5 of WO '230), WO '230 is directed to extracting PHA's, whereas the claimed invention is directed to extracting glucosinolates and isothiocyanates.



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PHA's are distinct from glucosinolates and isothiocyanates. Attached as Exhibit 1 is a description of polyhydroxyalkanoates<sup>1</sup> which illustrates the different characteristics of PHA's as compared to glucosinolates and isothiocyanates. PHA's are cross-linked polymers of much higher molecular weight than glucosinolates and isothiocyanates. PHA's are synthesized from low molecular weight organic acids and glucose, whereas glucosinolates and isothiocyanates are synthesized from amino acids. Glucosinolates are very polar, charged anionic species and isothiocyanates are highly reactive species, whereas PHA's are neither. Therefore, based upon the very different chemical structures, biosyntheses, reactivity, polarity, etc., of PHA's as compared to glucosinolates and isothiocyanates, a person of ordinary skill in the art would not have been motivated to use a method for extracting PHA's to extract glucosinolates and isothiocyanates and the references themselves fail to provide motivation to do so.

At page 4 of the outstanding Office Action, the Examiner states that motivation is provided because WO '230 states that the use of at least one solvent selected from the group consisting of acetone, acetonitrile, benzene, butyl acetate, butyl propionate, beta-butyrolactone, gamma-butyrolactone, diethyl carbonate, diethylformamide, dimethyl carbonate, dimethyl succinate, dimethyl sulfoxide, dimethylformamide, ethyl acetate, ethylene glycol diacetate, methyl acetate, methyl ethyl ketone, 1,4-dioxane, tetrahydrofuran, toluene, xylene, and mixtures thereof results in an environmentally friendly and economical process for recovering products from a large-scale biological source. However, as discussed above, because of the very different chemical structures, biosyntheses, reactivity, polarity, etc., of PHA's as compared to glucosinolates and isothiocyanates, a person of ordinary skill in the art would have had no reasonable expectation of success to use a method for extracting PHA's to extract glucosinolates and isothiocyanates, and the fact that the method is environmentally friendly and economical would not have overcome the issue.

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<sup>1</sup> Exhibit 1 is from [www.usask.ca/agriculture/plantsci/classes/plsc416/projects\\_2002/immell/polyhydroxyalkanoates.html](http://www.usask.ca/agriculture/plantsci/classes/plsc416/projects_2002/immell/polyhydroxyalkanoates.html).

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- iii. *There is no reasonable expectation of success in combining the teachings of Jones et al. and WO '230 to arrive at the claimed invention.*

Additionally, there is no reasonable expectation of success in combining the teachings of Jones et al. and WO '230 to arrive at the present invention. because WO '230 is directed to a method for separating polyhydroxyalkanoate (PHA) from a biomass and the present invention is directed to extracting glucosinolates and isothiocyanates from a plant tissue. At best, the examiner is using an improper "obvious to try" standard, arguing that it would have been obvious to a person of ordinary skill in the art familiar with the teachings of Jones et al. and WO '230 to try to extract glucosinolates and isothiocyanates from plant tissue using a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide. However, "'obvious to try' has long been held to not constitute obviousness." *In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210 (Fed. Cir. 1995).

Applicant notes that the Examiner failed to provide a reason as to why a person of ordinary skill in the art would be motivated to combine the teachings of Jones et al. and WO '230. See page 4, line 9 of the outstanding Office Action where the Examiner states "[s]uch a modification would have been motivated by the reasonable expectation that ???"

- b. Rejection of Claims 58, 59, 60, 62, 63, 65-67, 68-70 and 71 as Being Obvious Over Jones et al. (USP 4,158,656) in view of Pusateri et al. (USP 5,882,646) and Cho et al. (WO 94/19948).

Claims 58, 59, 60, 62, 63, 65-67, 68-70 and 71 are rejected by the Examiner under 35 U.S.C. § 103 as being obvious over Jones et al. (USP 4,158,656) in view of Pusateri et al. (USP 5,882,646) and Cho et al. (WO 94/19948). Applicant respectfully requests reconsideration and withdrawal of the rejection.

The claimed invention is not obvious over the combined teachings of Jones et al., Pusateri et al. and Cho et al. because Jones et al. explicitly teaches away from the claimed invention. As discussed in Applicant's July 16, 2003 response, at column 3, lines 48-53 of Jones et al. it states that "it is essential for food use, to remove the glucosinolates and those other factors that can cause unattractive flavor and coloration and decreased nutritive value of foods." In contrast, claim 58 (and dependent claims 59-63 and 65-71) is directed to a method

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of making a food product that involves adding extracted glucosinolates and isothiocyanates to food.

Applicant again reminds the Examiner that it is improper to take individual teachings from one reference out of context (Jones et al.) and to combine those individual teachings with teachings from other references (Pusateri et al. and Cho et al.) to make an obviousness rejection. Instead, the Examiner must consider Jones et al. in its entirety. As discussed above, Jones et al. explicitly teaches removing glucosinolates from food because of their deleterious properties, such as decreased nutritive value and unattractive flavor. Because Jones et al. teaches away from adding glucosinolates to food, the prior art would not have suggested to those of ordinary skill in the art that they should modify the Jones et al. method by adding the isolated glucosinolates to food products, as the Examiner alleges is suggested by Pusateri et al. and Cho et al. Additionally, because Jones et al. teaches away from adding glucosinolates to food, those of ordinary skill would not have a reasonable expectation of success in combining the teachings of Jones et al. with the teachings of Pusateri et al. and Cho et al.

- c. Rejection of Claim 61 as Being Obvious Over Jones et al. (USP 4,158,656) in View of Pusateri et al. (USP 5,882,646), Cho et al. (WO 94/19948) and Passey et al. (USP 5,290,578)

Claim 61 is rejected by the Examiner under 35 U.S.C. § 103 as being obvious over Jones et al. (USP 4,158,656) in view of Pusateri et al. (USP 5,882,646), Cho et al. (WO 94/19948) and Passey et al. (USP 5,290,578). Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 61 is not obvious over the combined teachings of Jones et al., Pusateri et al., Cho et al. and Passey et al. because Jones et al. teaches away from the claimed invention, as discussed above. It is improper to take individual teachings from one reference out of context (Jones et al.) and to combine those individual teachings with teachings from other references (Pusateri et al., Cho et al. and Passey) to make an obviousness rejection (see above discussion).

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**CONCLUSION**

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant(s) hereby petition(s) for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date November 14, 2005

FOLEY & LARDNER LLP

Customer Number:

**22428**

PATENT TRADEMARK OFFICE

Telephone: (202) 672-5483

Facsimile: (202) 672-5399

By *R. C. Peet, Reg. #29,768*

*R. C. Peet*  
Richard C. Peet  
Attorney for Applicant  
Registration 35,792

# Polyhydroxyalkanoates (PHAs)

PHAs make up a class of polymers that are fully biodegradable. They are a family of polyesters with a wide array of physical properties that can range from stiff-brittle plastics to elastomers to rubbers. PHAs are naturally produced in numerous genera of bacteria, and have been amplified through bacterial fermentation.

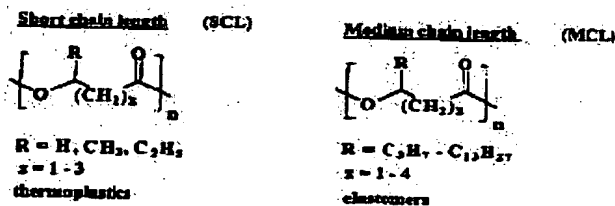
PHAs are mainly composed of R-(-)-3-hydroxyalkanoic acid monomers. There can be broadly subdivided into two groups:

## Short chain length PHAs

- consist of 3 carbon - 5 carbon monomers (C3-C5)
- produced by bacterium *Alcaligenes eutrophus* (plus others)

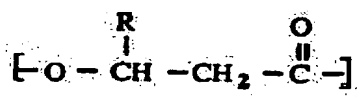
## Long chain length PHAs

- consist of 6 carbon - 14 carbon monomers (C6-C14)
- produced by *Pseudomonas oleovorans* (plus others)

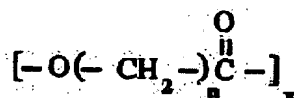


Each type of PHA generally consists of 1000-10000 monomers, but most are synthesized by short chain lengthed monomers.

There are many different types of PHAs, distinctly characterized by chain length, type of functional group and degree of unsaturated bonds. A higher degree of unsaturation increases the rubber qualities of a polymer, and different functional groups change the physical and chemical properties of a polymer.



R = hydrogen	3-hydroxypropionate	(3HP)
R = methyl	3-hydroxybutyrate	(3HB)
R = ethyl	3-hydroxyvalerate	(3HV)
R = propyl	3-hydroxycaproate	(3HC)
R = butyl	3-hydroxyheptanoate	(3HH)
R = pentyl	3-hydroxyoctanoate	(3HO)
R = hexyl	3-hydroxynonanoate	(3HN)
R = heptyl	3-hydroxydecanoate	(3HD)
R = octyl	3-hydroxyundecanoate	(3HUD)
R = nonyl	3-hydroxydodecanoate	(3HDD)



n = 3	4-hydroxybutyrate	(4HB)
n = 4	5-hydroxyvalerate	(5HV)

Figure scanned from Kumar & Minocha, In Transgenic Plant Research; Harwoo Pub.  
[www.routledge.com](http://www.routledge.com)

**PHB [or P(3HB)] is the most common type of PHA produced and is an example of a short chain lengthed homopolymer produced by *A. eutrophus*. PHB has poor physical properties for commercial use. It is stiff, brittle and hard to process. This has led to an increased interest to produce heteropolymers with improved qualities. P(3HB-3HV) is an example of an improved heteropolymer. It is named Biopol, and was produced by Zeneca, but now owned by Monsanto. Compared to PHB, P(3HB-3HV) is less stiff, tougher, and easier to process, making it more suitable for commercial production. It is also water resistant and impermeable to oxygen, increasing its value.**

**PHB is 100% biodegradable. Various bacteria and fungi degrade PHB to carbon dioxide and water through secreting enzymes. It can also be degraded through non-enzymatic hydrolysis. Degredation appears to be the fastest under conditions of high temperatures and mechanical disruption. An 80% loss of PHB has been degraded in 15 weeks. PHB is also biocompatible, meaning it is a metabolite normally present in blood.**

**Researchers have amplified the production of PHAs for large scale production, to produce and commercialize biodegradable plastics. Originally bacterial fermentation was used, but PHA can only be produced on a small scale this way, and production costs are too expensive compared to production of synthetic plastics. Researchers have since tried large scale production through transgenic plants, but many problems have arisen this way also. More research must be done before commercial production of PHAs will be able to outcompete or replace synthetic plastics.**

**references:**

**Imam, S., Greene, R. and Zaidi, B. 1999. Biopolymers, Utilizing Nature's Advanced Materials. American Chemical Society, Washington, DC. 111 p.**

**Lindsey, Keith. 1998. Transgenic Plant Research. Harwood Academic Publishers. Amsterdam, The Netherlands. 201-219 pp.**

**Poirier, Y., Nawrath, C. and Somerville, C. 1995. Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. Bio/Technology 13: 142-149.**

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Atty. Dkt. No. 046585-0138

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Jed FAHEY et al.

Title: CANCER CHEMOPROTECTIVE  
FOOD PRODUCTS

Appl. No.: 09/825,989

Filing Date: 4/5/2001

Examiner: Delacroix-Muirheid, Cybille

Art Unit: 1614

**AMENDMENT TRANSMITTAL**Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is an Amendment and Exhibit 1 in the above-identified application.

☒ [ X ] Small Entity status under 37 C.F.R. § 1.9 and § 1.27 has been established by a previous assertion of Small Entity status.☒ [ X ] The fee required for additional claims is calculated below:

	Claims As Amended		Previously Paid For		Extra Claims Present		Rate		Additional Claims Fee
Total Claims:	23	-	24	=	0	x	\$50.00	=	\$0.00
Independent Claims:	2	-	3	=	0	x	\$200.00	=	\$0.00
First presentation of any Multiple Dependent Claims:						+	\$360.00	=	\$0.00
CLAIMS FEE TOTAL									\$0.00



Atty. Dkt. No. 046585-0138

- ☒ Applicant hereby petitions for an extension of time under 37 C.F.R. §1.136(a) for the total number of months checked below:

<input checked="" type="checkbox"/> Extension for response filed within the first month:	\$120.00	\$120.00
<input type="checkbox"/> Extension for response filed within the second month:	\$450.00	\$0.00
<input type="checkbox"/> Extension for response filed within the third month:	\$1,020.00	\$0.00
<input type="checkbox"/> Extension for response filed within the fourth month:	\$1,590.00	\$0.00
<input type="checkbox"/> Extension for response filed within the fifth month:	\$2,160.00	\$0.00
EXTENSION FEE TOTAL:		\$120.00
<input type="checkbox"/> Statutory Disclaimer Fee under 37 C.F.R. 1.20(d):	\$130.00	\$0.00
CLAIMS, EXTENSION AND DISCLAIMER FEE TOTAL:		\$120.00
<input checked="" type="checkbox"/> Small Entity Fees Apply (subtract ½ of above):		\$60.00
TOTAL FEE:		\$60.00

- ☒ A check in the amount of \$60.00 is enclosed.

- ☒ The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date November 14, 2005  
 FOLEY & LARDNER LLP  
 Customer Number: 22428  
 Telephone: (202) 672-5483  
 Facsimile: (202) 672-5399

By *Richard C. Peet* Reg # 29,768  
 for Richard C. Peet  
 Attorney for Applicant  
 Registration No. 35,792

## PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 2000

Application or Docket Number

09/825,989

## CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	20	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	20 minus 20 =	0
INDEPENDENT CLAIMS	2 minus 3 =	0
MULTIPLE DEPENDENT CLAIM PRESENT		<input type="checkbox"/>

\* If the difference in column 1 is less than zero, enter "0" in column 2

## CLAIMS AS AMENDED - PART II

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	24	4	4
Independent	2	...	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			<input type="checkbox"/>

4.19.05

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	23	24	-
Independent	2	3	-
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			<input type="checkbox"/>

11-14-05

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total			=
Independent			=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			<input type="checkbox"/>

\* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

\*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."

\*\*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

SMALL ENTITY TYPE ☒

OR OTHER THAN SMALL ENTITY

RATE	FEE
BASIC FEE	355.00
X\$ 9=	
X40=	
+135=	
TOTAL	355

RATE	FEE
BASIC FEE	710.00
X\$18=	
X80=	
+270=	
TOTAL	

SMALL ENTITY OR

OTHER THAN SMALL ENTITY

RATE	ADDI-TIONAL FEE
X\$ 9=	36.00
X40=	
+135=	
TOTAL	36.00

RATE	ADDI-TIONAL FEE
X\$18=	
X80=	
+270=	
TOTAL	

RATE	ADDI-TIONAL FEE
X\$ 9=	
X40=	
+135=	
TOTAL	

RATE	ADDI-TIONAL FEE
X\$18=	
X80=	
+270=	
TOTAL	

RATE	ADDI-TIONAL FEE
X\$ 9=	
X40=	
+135=	
TOTAL	

RATE	ADDI-TIONAL FEE
X\$18=	
X80=	
+270=	
TOTAL	

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=> que glucosinolate  
L1 QUE GLUCOSINOLATE  
  
=> que isothiocyanate  
L2 QUE ISOTHIOCYANATE  
  
=> que l1 and l2  
L3 QUE L1 AND L2  
  
=> que plant or vegetable  
L4 QUE PLANT OR VEGETABLE  
  
=> que extract?  
L5 QUE EXTRACT?  
  
=> que l3 and l4 and l5  
L6 QUE L3 AND L4 AND L5  
  
=> s l6  
2345 GLUCOSINOLATE  
24461 ISOTHIOCYANATE  
805781 PLANT  
87635 VEGETABLE  
299930 EXTRACT?  
L7 10 L3 AND L4 AND L5

	Type	L #	Hits	Search Text	DBs
1	BRS	L1	611	glucosinolate	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
2	BRS	L2	156	glucosinolate and isothiocyanate	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
3	BRS	L3	140	glucosinolate and isothiocyanate and (plant or vegetable)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
4	BRS	L4	120	glucosinolate and isothiocyanate and (plant or vegetable) and (extract or extracted or extracting)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T

	Type	L #	Hits	Search Text	DBs
5	BRS	L5	24	glucosinolate and isothiocyanate and (plant or vegetable) and (extract or extracted or extracting) and (chemoprevent\$ or chemoprotect\$)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T

	Type	L #	Hits	Search Text	DBs
1	BRS	L1	611	glucosinolate	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
2	BRS	L2	156	glucosinolate and isothiocyanate	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
3	BRS	L3	140	glucosinolate and isothiocyanate and (plant or vegetable)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T
4	BRS	L4	120	glucosinolate and isothiocyanate and (plant or vegetable) and (extract or extracted or extracting)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T

	Type	L #	Hits	Search Text	DBs
5	BRS	L5	24	glucosinolate and isothiocyanate and (plant or vegetable) and (extract or extracted or extracting) and (chemoprevent\$ or chemoprotect\$)	US- PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWEN T